

649-Pos**Diverse Effects of a Benzofuroindole on Different K⁺ Channels and Localization of Its Receptor on BK_{Ca} Channel**

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Gwangju institute of science and technology, Gwangju, Korea, Republic of. We reported previously that the activity of the large-conductance calcium-activated potassium channels (BK_{Ca} channel) could be strongly potentiated by certain derivatives of benzofuroindole scaffold when treated from extracellular side of the membrane (Gormemmis *et al.*, 2005; Ha *et al.*, 2006). In order to localize the receptor site on the BK_{Ca} channel, we surveyed the effects of CTBIC, the most potent benzofuroindole compound, on various K⁺ channels. While the compound increase the activity of voltage-gated K⁺ channels, K_v1.5 and HERG, CTBIC did not affect the activity of inward rectifier K⁺ channel, Kir3.1, significantly. Intriguingly enough, the same compound greatly decreased the activity of SK2, a different subclass of Ca²⁺-activated K⁺ channel. In addition, the affinity of charybdotoxin, a peptide pore-blocker, was reduced by the co-treatment with CTBIC, whereas that of tetraethylammonium, a small pore-blocking quaternary ammonium, was not altered. Guided by these results, we performed mutagenesis studies on the outer vestibule of the BK_{Ca} channel to localize the residues that affect the binding of CTBIC. We identified three residues in the loop that connects with the pore-forming region of the channel, which was strongly affected by alanine substitution. Our results suggest that the turret region of the BK_{Ca} channel may play a critical role in the modulation of the channel activity and may thus represent a therapeutic target site of K⁺ channels.

650-Pos**NS8593-Mediated Negative Gating Modulation Depends on Residues in the Inner Pore Vestibule of Kca2 Channels**David P. Jenkins¹, Charlotte Hougaard², Marianne L. Jensen², Rene Hummel², Ulrik Sørensen², Heike Wulff¹, Palle Christophersen², Dorte Strøbæk².¹University of California, Davis, CA, USA, ²NeuroSearch A/S, Ballerup, Denmark.

The identification of NS8593 has provided a selective and novel means for modulating the activity of the small-conductance calcium-activated potassium channels K_{Ca}2.1-2.3. Acting as a negative gating modulator, NS8593 shifts the apparent calcium dependence of channel gating to higher calcium concentrations. It has been assumed that the binding site for NS8593 was located in the C-terminal region, similar to that of some positive gating modulators (e.g. EBIO and CyPPA, but not GW542573X). However, by employing a progressive chimera approach, (where all critical constructs were tested for normal Ca²⁺-sensitivity in inside-out patches) we were able to localize the site-of-action to the pore. For example, when we transferred the C-terminus from the NS8593-insensitive intermediate-conductance K_{Ca}3.1 channel to K_{Ca}2.3 the chimeric channel remained as sensitive to NS8593 as WT-K_{Ca}2.3. In contrast, when we transferred the K_{Ca}2.3 pore, K_{Ca}3.1 became sensitive to NS8593. Subsequently, by using site-directed mutagenesis we identified two residues in the inner vestibule of K_{Ca}2.3 (Ser-507 and Ala-532) that mediate the activity of NS8593. By mutating these residues to the corresponding residues in K_{Ca}3.1 (Thr-250 and Val-275), we were able to make K_{Ca}2.3 insensitive. Conversely, replacement of these two residues was sufficient to render K_{Ca}3.1 sensitive to NS8593. The positions of these residues, Ser-507 in the pore-loop near the selectivity filter and Ala-532 in an adjacent position in the S6 segment, are within in the region predicted to contain the channel gate. Based on these results, we propose that NS8593 mediated gating modulation of K_{Ca}2.3 occurs at a position deep within the inner pore vestibule.

651-Pos**Structural Determinant of Altered Current Expression, Activation Kinetics and Beta-Subunit Interaction of the Neuronal X1 Splice Variant of the Rat BK Channel**Asser N. Poulsen¹, Inger Jansen-Olesen², Jes Olesen², Dan Klaerke¹.¹Faculty of Life Sciences, Univ. of Copenhagen, Frederiksberg C, Denmark,²The Danish Headache Center, Glostrup Hospital, University of Copenhagen., 2600 Glostrup, Denmark.

We have identified and cloned a splice variant of the rat BK channel called X1 (Poulsen *et al.*, 2009, *Biochimica et Biophysica Acta*. 1788(380-389)) which is exclusively expressed in brain or nervous tissue, which has not previously been functionally characterized. The X1 variant is different from the insert-less variant Zero by having an eight amino acid insert in the extracellular loop between S1 and S2, a four amino acid insert between C-terminal S8 and S9 (SS1) and 27 amino acids between S9 and S10. Another variant

Slo27, widely expressed in brain and some vascular tissues, also contains the 27 residues between S9 and S10 but only a 3 residue insert between S8 and S9 (SS2). When expressed in *Xenopus* oocytes, the X1 variant shows less current expression than Slo27 or Zero and an apparently faster activation speed. We attempted to dissect the underlying mechanism by generating constructs lacking one of the insert sequences. Deletion of the eight amino acids between S1 and S2 resulted in higher current expression similar to Slo27 or Zero while retaining the fast activation speed. Deletion of the four S8-S9 residues resulted in low current expression but still fast activation. Thus the eight residue insert seems to suppress channel surface expression or channel gating at low calcium concentrations, while the structural determinant of fast activation speed is less clear.

We also co-expressed the X1 variant with beta 2, which is present in nervous tissue also. Beta 2 co-expression reduced current expression further and slowed channel activation but showed no signs of inactivation (at low calcium), which is a key feature of beta 2 when co-expressed with Zero or Slo27.

652-Pos**Acceleration of Cutaneous Wound Healing by Suppression of Large Conductance Ca²⁺-Activated K⁺ Channels**

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Many kinds of K⁺ channels are involved in the regulation of cell migration and proliferation, which are required for the processes of wound healing. However, the role of K⁺ channels on cutaneous wound healing has not yet been reported. Here, we demonstrate that inhibition of large conductance Ca²⁺-activated K⁺ (BK_{Ca}) channels expressed in human epidermal keratinocyte facilitate cutaneous wound healing by activating both cell migration and proliferation. In the group treated with 25 mM KCl, *in vivo* wound healing was facilitated more rapidly than that in control group. *In vitro* assay of wound healing showed that 25 mM KCl significantly increased wound closure in keratinocytes after creation of linear wound with ~200 μ m wide defect. KCl (25 mM) promoted processes of cell migration and proliferation. BK_{Ca} and two-pore domain K⁺ channels were recorded in the keratinocytes by using patch-clamp technique. The BK_{Ca} channel, among these K⁺ channels, is the most frequently observed in cell-attached mode. NS1619, a BK_{Ca} channel opener, inhibited the proliferation and migration of keratinocytes in a dose- and time-dependent manner. Charybdotoxin and iberiotoxin, BK_{Ca} channel blockers, facilitated both cell proliferation and migration by 10 \pm 7% and 30 \pm 4%, respectively. Cutaneous wound healing was also facilitated by siRNA against BK_{Ca} (BK_{Ca}/siRNA). The migration and proliferation were more enhanced by cotransfection with BK_{Ca}/siRNA and TASK-1/siRNA. BK_{Ca} channel blockers activated PKC and ERK in a time-dependent manner. These results show that BK_{Ca} and TASK-1 channels regulate proliferation and migration of human epidermal keratinocytes by activation of PKC-ERK pathway and indicate that BK_{Ca} channel could be a molecular target for regulation of cell proliferation and migration.

653-Pos**An Unconventional Role in Store-Independent Constitutive Calcium Signaling by the Secretory Pathway Calcium - ATPases in Mammary Tumors**Mingye Feng¹, Desma Grice², Helen Faddy², Nguyen Nguyen¹, Sharon Leitch¹, Sabina Muend¹, Paraic Kenny³, Sara Sukumar¹, Sarah Roberts-Thomson², Gregory Monteith², Rajini Rao¹.¹Johns Hopkins University, School of Medicine, Baltimore, MD, USA, ²The University of Queensland, Brisbane, Australia, ³Albert Einstein College of Medicine, Bronx, NY, USA.

Constitutive calcium signaling in cancer cells drives tumor proliferation and metastasis. Secretory Pathway Ca²⁺-ATPases (SPCA) were highly upregulated in breast cancer derived cell lines and human breast tumors. Depletion of SPCA in human breast adenocarcinoma cells attenuated basal Ca²⁺ levels and downstream cell proliferation, anchorage-independent growth and tumor formation in mice. Contrary to its known role in Golgi Ca²⁺ sequestration, SPCA overexpression increased cytosolic Ca²⁺ by activation of the store-operated Ca²⁺ channels. However, SPCA mediated Ca²⁺ influx was independent of Ca²⁺ stores or sensors and not dependent on its transport ATPase activity, revealing a new signaling paradigm.

654-Pos**Role Of Ca²⁺-Activated K⁺ Channel in the Neurogenic Contractions Induced by Electrical Field Stimulation in Detrusor Smooth Muscle Isolated from Rats and Guinea Pigs**

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